Cadmium Burden of Men and Women Who Report Regular Consumption of Confectionery Sunflower Kernels Containing a Natural Abundance of Cadmium

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Because of inherent genetic and physiological characteristics, the natural concentration of cadmium in the kernels of sunflowers grown in uncontaminated soils of the northern Great Plains region of the United States is higher than in most other grains. We tested the hypothesis that a habitual consumption of sunflower kernels will increase the body burden and health effects of cadmium in humans. Sixty-six men and women who reported consuming various amounts of sunflower kernels were recruited and divided by sex and kernel consumption: those who consumed less than or equal to 1 ounce(oz)/week and those who consumed more than 1 oz/week. Cadmium intake was assessed by calculation from 7-day food diaries, cadmium burden by whole blood cadmium, red blood cell (RBC) cadmium and urine cadmium concentrations, and health effects by urinary excretion of *N*-acetyl-β-D-glucosaminidase (NAG) activity and β_2-microglobulin (β2MG). The results showed that high intakes of sunflower kernels (>1 oz/day) significantly increased the intake of cadmium ($p<0.004$). However, the amount of cadmium in whole blood or RBCs was not affected by cadmium intake. Urinary excretion of cadmium also was not affected by cadmium intake. Urine NAG activity and the amount of urinary β2MG were significantly elevated in the urine of high sunflower kernel consumers when the values were expressed on a urine volume basis ($p<0.03$), but not when expressed on a creatinine basis ($p>0.05$). Because normal ranges for the excretion of these protein markers have not been established, it was not possible to determine if these elevated values were meaningful. However, given the knowledge that habitual consumption of sunflower kernels with natural cadmium concentrations higher than most other food products will increase the average intake of dietary cadmium, the potential exists for an increased body burden of cadmium. Controlled feeding studies in humans should be pursued in order to determine if the body burden does indeed increase and, if so, is it a cause for concern. *Key words*: cadmium, confectionery sunflower kernels, copper, dietary intake, ferritin, humans, iron, β_2-microglobulin, *N*-acetyl-β-D-glucosaminidase, zinc.
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Cadmium intake in relatively high amounts can be detrimental to human health. Over a long period of continuous intake, cadmium may accumulate in the kidney and liver and, because of its long biological half-life, may lead to kidney damage. Food is the major source of cadmium intake in the United States, and cadmium is present in most foods in low concentrations. The average daily lifetime intake of cadmium in adult males in the United States has been estimated at 10–20 µg, or 140 µg/week (*1,2*).

Because of inherent genetic and physiological characteristics, some food crops, including confectionery sunflowers, have a propensity to take up cadmium from the soil in which they are grown and deposit it in the kernels. As a consequence, the sunflower kernel has a higher natural concentration of cadmium than most other grains, even when grown on uncontaminated soils (*3*).

The concentration of cadmium in confectionery sunflower kernels may range from 0.2 to 2.5 µg/g fresh weight, depending on the genotype, the seasonal conditions, and the local soil conditions in the area where the sunflowers are grown (*3,4*). Our laboratory analyzed 19 different lots of sunflower kernels from the 1995 crop grown in the northern Great Plains region of North Dakota and Minnesota and found a range of 0.33–0.67 µg/g, with a mean ± standard deviation (SD) of 0.48 ± 0.11 µg/g fresh kernels. As a result of the relatively high cadmium content, habitual consumption of the kernels could substantially increase the daily intake of cadmium.

Guidelines proposed by the World Health Organization (WHO) recommend a provisional tolerable weekly intake (PTWI) of 490 µg cadmium for a person who weighs 70 kg (*5*). A consistent consumption of 1 ounce (oz) of sunflower kernels per day could increase the average consumption of total cadmium by about 70%, or to about 235 µg/week. This is still far below the PTWI, but higher intakes of kernels could increase the average intake even further. Would this, in turn, increase the cadmium burden of the consumer, and what would be the consequences if it did?

The following study was designed to answer these questions. This study was approved by the Institutional Review Board of the University of North Dakota and the Human Studies Committee of the U.S. Department of Agriculture (USDA). The protocol followed the guidelines of the U.S. Department of Health and Human Services and the Helsinki Doctrine regarding the use of human subjects.

Materials and Methods

Study group. One hundred twenty-five men and women between 30 and 70 years of age who lived the Red River Valley Region of North Dakota and Minnesota were recruited by newspaper ads and flyers. The initial selection was made based on a medical history questionnaire and a food frequency questionnaire that emphasized the consumption of sunflower kernels in a typical Western diet. Certain dietary habits and medical conditions prohibited volunteers from participating in the study. These included severe weight control diets, kidney and urological disorders, diabetes mellitus, hypertension, liver and pancreatic disorders, gastrointestinal disorders, infections in the previous 4 weeks, or intakes of drugs known to affect renal function.

Based on their responses to the food frequency survey, the volunteers were arbitrarily

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subdivided into two categories: 1) those who reported consuming ≤1 oz sunflower kernels/week for the past year, and 2) those who reported consuming >1 oz sunflower kernels/week for the past year. Seventy-five of the recruits were chosen to complete the study, including 44 women and 31 men. However, because smoking tends to affect cadmium status, data for all smokers, 6 women and 3 men, were removed before the final analysis. The volunteers were free-living and consumed a normal self-selected diet throughout the study.

Sample collections and food diaries. The volunteers were invited to an informational meeting before the study began and were given a detailed explanation of what the study was about, what type of data we expected to obtain from their participation, and their rights and obligations if they decided to participate. Each volunteer who decided to participate in the study signed a statement of consent.

The volunteers were given instructions on how to collect urine and fecal samples and were trained by a registered dietitian to keep a detailed 7-day food diary. They were instructed, with emphasis, that they should refrain from changing their usual dietary habits during the diary period. After completion of the food diary, each participant was given an extensive interview by a dietitian to determine the accuracy of reporting. Individual items in the diary were then analyzed for nutrient content by using a computerized nutrient database called Grand Forks Research Analysis of Nutrient Data (GRAND). It is based in part on the online version of the USDA Handbook No. 8 and updated with analyzed values for the cadmium content of 260 different types of food [FDA Total Diet Study, Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA), Washington, D.C.]. The data were expressed as the amount of nutrient intake per day or amount per 10 megajoules (MJ).

The GRAND database tends to overestimate the concentrations of food minerals such as zinc, copper, iron, and calcium, and underestimates the content of cadmium. To compensate for this imprecise information, the mineral values generated from the database were corrected with a factor generated by chemical analyses of individual daily duplicate diets over a period of 7 days. Separate components of duplicate diets for one male volunteer were collected and carefully weighed to the nearest 0.1 g. All components of each daily diet were mixed together with deionized water and homogenized into a slurry in a large blender. The total slurry was weighed and an aliquot lyophilized to constant weight and analyzed for zinc, copper, iron, calcium, and cadmium. The kind and amount of individual components of the daily intakes were carefully recorded and then analyzed by using the GRAND database. The mean ratio of the difference between the chemical analysis and the database analysis of individual daily diets was then used to correct the database values generated from the food diary of each of the other volunteers. Daily intake values were expressed as the mean ± SD of seven individual daily diet periods.

At the end of the food diary period, each volunteer provided two overnight urine samples; overnight was defined as the time the volunteer normally slept. Urine was collected at 4°C in thermos-type containers and analyzed within 4 hr of the last void for the concentrations of *N*-acetyl-β-D-glucosaminidase (NAG) (*6*), β_2-microglobulin (β2MG; Abbott Laboratories, Abbott Park, IL) and creatinine (UC; Roche Diagnostic Systems, Nutley, NJ). The remaining urine was frozen for future analysis of cadmium content. Values for all urine measurements were expressed as the mean of the two collections. During the last part of the food diary period, each volunteer provided a 5-day fecal collection for the analysis of cadmium. At the end of the diary period, and after a 12-hr fast, blood was drawn from each volunteer via the brachial vein into two tubes; one was heparinized to obtain whole blood and red blood cells and one tube was used to obtain serum. Hemoglobin concentration was determined in whole blood; cadmium concentration in washed red blood cells (RBCs); and iron, ferritin (Abbott Laboratories, Abbott Park, IL), zinc, and copper were measured in serum.

Mineral determinations. RBCs were separated from whole blood and washed three times in isotonic Tris buffer, pH 7.4. Triplicate, one-half gram samples of packed cells were digested in 3.0 ml concentrated HNO_3 (J.T. Baker, Instra-Analyzed, Phillipsburg, NJ) at 145°C in Teflon tubes. When the sample had boiled dry, 1.5 ml 30% H_2O_2 was added to completely oxidize the organic residue. When the H_2O_2 had evaporated, the mineral residue was dissolved in 2 ml 5% HNO_3 and analyzed for cadmium by graphite furnace atomic absorption (GFAA) spectrometry using Zeeman background correction.

Serum was diluted 1:5 with deionized water and analyzed directly for iron, zinc, and copper by inductively coupled argon plasma emission spectrometry (ICAP).

Urine samples were analyzed for cadmium by GFAA without further processing and without dilution.

Diet and feces samples were lyophilized to constant weight. One and one-half grams of diet or 0.3 g feces was digested with 5 ml HNO_3–$HClO_4$ (6:1 vol/vol; J.T. Baker, Instra-Analyzed) in Teflon tubes at 145°C until all organic material was removed. The ash residue from diet samples was diluted appropriately with 5% HNO_3 and analyzed for iron, zinc, copper, and calcium by ICAP and for cadmium by GFAA. Fecal ash residue was treated similarly and analyzed for cadmium by GFAA.

GFAA analysis. The GFAA instrument was equipped with a stabilized temperature platform furnace with Zeeman background correction (Zeeman GFAA Spectrometer, Model 3030, Perkin-Elmer Co., Norwalk, CT). An electrodeless-discharge lamp was used with a spectral bandwidth of 0.7 nm. Peak area measurements were taken by using pyrolytic graphite tubes with graphite platforms inserted. The matrix modifier used for each type of sample was a 0.20% solution of NH_4NO_3. The automatic sampler was programmed to deliver 15 µl sample followed by 5 µl NH_4NO_3 solution. The sample was then heated to dryness before charring and atomization. Cadmium was detected by using a spectral line of 228.8 nm. See Table 1 for a more detailed account of the analytical conditions for cadmium analysis.

Quality control. All containers used for sample collection and sample analysis were tested for mineral contamination before use. Quality control samples of bovine liver (#1577b; National Institute of Standards and Technology, Gaithersburg, MD) and serum (#66816; UTAK Laboratories, Inc., Valencia, CA) with known concentrations of

Table 1. Analytical conditions for cadmium analysis by Zeeman GFAA spectrometer

Steps[a]	Event	Furnace temperature (°C)	Ramp time (sec)	Hold time (sec)
1	Drying	90	20	20
2	Drying	150	20	20
3	Drying	250	20	20
4	Charring	900	10	20
5	Atomization[b]	1,700	0	4
6	Cool Down	20	1	3
7	Clean out	2,200	3	3

[a]This series of events was initiated as described in Materials and Methods.
[b]Peak area was measured at 228.8 nm.

minerals were run with each batch of serum, red cells, diet, and fecal samples. Quality control samples of urine (UTAK Laboratories, Inc.) were run with each batch of urine samples.

Statistical analysis. The data were statistically analyzed with analysis of variance (ANOVA) as a 2 × 2 factorial, with sex and reported sunflower kernel intake as the two factors. When data did not meet the criteria for normal distribution, the ANOVA was performed on natural log (ln) transformed data. The Tukey (*7*) post-hoc test was run to determine differences between means when a significant interaction was observed. The ln transformed means were back-transformed and listed in the tables. Because ln transformed data are not linear, the SD cannot be back-transformed; therefore, a back-transformed SD range was given for each mean. Values for all other parameters were expressed as means ± SD.

Results

Quality control. Quality control standards that were run for all types of samples analyzed in this study fell within the range of acceptability. Table 2 shows the values of the quality control standards for cadmium. Analyzed values were within the ranges given for each reference material, with the coefficient of variation less than 6% for all standards except normal urine, where the cadmium values were extremely low and one would expect to have more variability. Therefore, the accuracies of cadmium measurements in the quality control standards were such that we can state with reasonable certainty that values obtained for each type of sample represented the true value for that sample.

Personal characteristics of volunteers. Table 3 shows the personal characteristics of the volunteers. Of the women volunteers, 29 reported consuming ≤1 ounce of sunflower kernels/week for the preceding year, and 9 consumed >1 oz/week. Of the men, there were 20 in the low consumption group and 8 in the high consumption group. None of the women in the high group reported consuming greater than 1 oz/day; however, 2 reported consuming 5 to 6 oz/week. Five out of 8 men reported consuming >1 oz/day, and 2 of these men reported consuming >4 oz sunflower kernels/day. There were no significant differences in age or body mass index (BMI) with respect to sex or to sunflower kernel consumption. However, the women in the high consumption group tended to weigh more than those in the other group, but the difference was not significant ($p>0.08$)

Table 4 shows the intake of calories, protein, and minerals as affected by sex and sunflower intake. As expected, men consumed more calories than women, but when expressed on a body weight basis, the difference disappeared (data not shown). Likewise, when expressed as intake per day, men consumed significantly more iron, zinc, and calcium than women ($p<0.05$). However, when expressed on a caloric basis, there were no differences between sexes. On the other hand, sunflower kernels contain higher amounts of copper than many foods, and this was reflected in a significant increase in copper intake for those volunteers who consumed >1 oz kernel/week ($p<0.001$). This was especially true for men. Even when expressed on a caloric basis, those who consumed high amounts of sunflower kernels had significantly higher intakes of copper ($p<0.006$).

The mineral concentrations in Table 4 were derived from values generated from a nutrient database and then adjusted with a correction factor obtained by actual chemical analyses of duplicate diets. Table 5 shows the calculated and analyzed values for the same dietary ingredients over a 7-day period. Compared with actual analysis, the database calculations overestimated the dietary content of iron, zinc, copper, and calcium by 15–20%, while cadmium was underestimated by about 50%. The 7-day mean of the ratios of differences between analyzed and calculated values were used to correct the calculated mineral values for each volunteer.

Both sex and intake of sunflower kernels

Table 2. Cadmium values for quality control reference standards

Reference standard	References values	Number[a]	Analyzed Cd ± SD	CV
Serum control	0.2–0.4 µg Cd/l	6	0.35 ± 0.02	5.8
Bovine liver	500 ± 30 µg Cd/kg	6	506 ± 18	3.5
Normal urine control	0.0–0.2 µg Cd/l	8	0.17 ± 0.02	13.0
High urine control	4.0–5.0 µg Cd/l	8	4.9 ± 0.2	4.3

Abbreviations: SD, standard deviation; CV, coefficient of variation.
[a]Values for serum and bovine liver represent six different batches run in triplicate over a 4-month period. Values for urine represent eight different batches run in triplicate over a 5-month period.

Table 3. Characteristics of volunteers[a]

	Frequency of sunflower kernel consumption[b]						
	Women		Men		*p*-Value determined by ANOVA		
	≤1 oz/week	>1 oz/week	≤1 oz/week	>1 oz/week	Sex	Group	Sex × group
Number	20	9	20	8			
Age (years)	45 ± 10	45 ± 11	46 ± 11	37 ± 6	NS	NS	NS
Weight (kg)	72.1 ± 15.5	80.9 ± 19.9	89.5 ± 17.1	94.8 ± 15.3	<0.002	NS	NS
Height (cm)	162 ± 6	165 ± 6	177 ± 6	182 ± 10	<0.001	NS	NS
BMI[c]	27.2 ± 5.2	30.0 ± 8.1	28.3 ± 4.7	29.0 ± 5.6	NS	NS	NS

Abbreviations: ANOVA, analysis of variance; NS, not significant; BMI, body mass index.
[a]Values are mean ± standard deviation.
[b]Frequency of sunflower kernel consumption was assessed by the response of each volunteer to a food frequency questionnaire.
[c]BMI is calculated by dividing the body weight in kilograms by the height in meters squared.

Table 4. Daily intake of calories, protein, iron, zinc, copper, and calcium calculated from a 7-day food diary[a]

	Frequency of sunflower kernel consumption[b]						
	Women		Men		*p*-Value determined by ANOVA		
	≤1 oz/week	>1 oz/week	≤1 oz/week	>1 oz/week	Sex	Group	Sex × group
Number	29	9	20	8			
Calories (MJ)	8.1 ± 1.7	8.5 ± 1.9	10.1 ± 2.0	11.7 ± 3.2	<0.001	NS	NS
Protein (MJ%)	14.5 ± 2.4	15.7 ± 2.3	14.6 ± 1.7	14.5 ± 3.0	NS	NS	NS
Fe (mg/day)	11.2 ± 2.6	11.9 ± 3.2	15.9 ± 6.7	13.9 ± 5.0	<0.01	NS	NS
Fe (mg/10 MJ)	17.9 ± 4.3	17.8 ± 3.6	19.8 ± 7.4	14.7 ± 2.9	NS	NS	NS
Zn (mg/day)	8.3 ± 2.4	9.3 ± 2.2	12.0 ± 4.7	12.7 ± 3.6	<0.001	NS	NS
Zn (mg/10 MJ)	12.5 ± 2.9	13.3 ± 2.6	14.3 ± 4.6	13.0 ± 1.8	NS	NS	NS
Cu (mg/day)	0.96 ± 0.24	1.08 ± 0.26	1.18 ± 0.33	1.67 ± 0.51	<0.001	<0.001	<0.03
Cu (mg/10 MJ)	1.20 ± 0.19	1.34 ± 0.51	1.17 ± 0.19	1.44 ± 0.21	NS	<0.006	NS
Ca (mg/day)	633 ± 285	654 ± 187	855 ± 219	716 ± 267	<0.05	NS	NS
Ca (mg/10 MJ)	925 ± 376	909 ± 207	1,004 ± 207	706 ± 144	NS	NS	NS

Abbreviations: ANOVA, analysis of variance; NS, not significant.
[a]Values are mean ± standard deviation.
[b]Frequency of sunflower kernel consumption was assessed by the response of each volunteer to a food frequency questionnaire.

Table 5. Comparison of database and chemical analyses of daily intakes of minerals from duplicate diets

Day	Cadmium (µg/day)		Iron (mg/day)		Zinc (mg/day)		Copper (mg/day)		Calcium (g/day)	
	Calculated[a]	Analyzed[b]	Calculated	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated	Analyzed
1	19.1	44.3	15.1	13.4	10.8	7.2	1.44	1.80	1.21	1.07
2	14.2	32.2	21.2	23.3	14.2	14.0	2.18	1.40	1.00	0.82
3	14.7	34.6	17.5	7.7	11.1	8.4	1.60	1.01	1.12	0.89
4	39.8	67.9	15.1	9.8	10.1	6.3	2.36	1.24	1.27	1.04
5	21.7	55.5	32.2	36.4	16.5	15.4	2.01	1.29	1.73	1.25
6	28.2	63.6	18.0	13.7	10.5	9.9	2.27	2.13	0.66	0.67
7	33.1	95.9	16.7	10.5	13.4	12.8	1.92	1.64	1.71	1.50
Mean ± SD	24.4 ± 12.4	56.3 ± 28.6	19.4 ± 8.8	16.4 ± 11.0	12.4 ± 4.9	10.6 ± 4.9	2.00 ± 0.77	1.50 ± 0.64	1.24 ± 0.56	1.03 ± 0.45

SD, standard deviation.
[a]Values were calculated from a food nutrient database (GRAND) maintained at the Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota. Values for the GRAND database are updated periodically from actual in-house analyses and from other reliable sources.
[b]All minerals were analyzed in ashed samples of daily composites of individual dietary components.

affected the daily amount of cadmium consumed (Table 6). Data for both methods of expression of cadmium intake did not fit the criteria for normal distribution; therefore, the statistical analysis was performed on the ln transformed data. The analysis revealed that volunteers who ate high amounts of kernels were more likely to have a high cadmium intake than those who ate small amounts of kernels ($p<0.004$). When the cadmium intake was expressed on a caloric basis, there was still a significant difference in cadmium intake between those who consumed different amounts of sunflower kernels ($p<0.04$).

Because cadmium is not absorbed well from the gut, the amount of cadmium in the feces usually reflects the amount in the diet. However, in this study, there was no correlation between cadmium intake and fecal cadmium (Table 6; correlation not shown). Men excreted significantly ($p<0.01$) more cadmium in the feces than women.

Values for blood and serum analyses are shown in Table 7. The values for whole blood cadmium were calculated based on the actual analysis of washed red cells and the value of individual hematocrit. Cadmium concentrations in whole blood and red blood cells were not significantly affected by eating large amounts of sunflower kernels. Calculated whole blood but not RBC cadmium was significantly higher in men than women ($p<0.04$). Typically, hematocrit, hemoglobin, ferritin, and serum iron concentrations were higher in men than women, but there were no significant differences caused by kernel intake. There were no significant differences in serum zinc concentrations between sex or sunflower kernel intakes. Although the apparent intake of copper was elevated in volunteers who consumed the higher amount of sunflower kernels, this was not reflected in a change in the concentrations of serum copper; however, women had significantly higher serum copper than men ($p<0.04$). Serum urea nitrogen and creatinine concentrations were within normal ranges and were unaffected by sunflower kernel consumption (Table 7).

Analysis of cadmium concentration in urine (Table 8) showed no significant effect of sunflower kernel intake; however, the activity of urinary NAG, used to monitor kidney dysfunction and expressed as U/l, was significantly higher in volunteers consuming

Table 6. Daily intake of dietary cadmium and the concentration of cadmium in feces[a]

	Frequency of sunflower kernel consumption[b]				*p*-Value determined by ANOVA		
	Women		Men				
	≤1 oz/week	>1 oz/week	≤1 oz/week	>1 oz/week	Sex	Group	Sex × group
Number	29	9	20	8			
Dietary Cd (µg/day)[c]	30 (18–50)	37 (21–63)	37 (24–55)	68 (43–108)	<0.004	<0.004	NS
Dietary Cd (ln µg/MJ)[c]	31 (18–54)	36 (19–67)	30 (19–46)	49 (28–84)	NS	<0.04	NS
Fecal Cd (µg/day)	21.5 ± 10.8	25.9 ± 13.4	33.5 ± 17.5	34.9 ± 17.6	<0.01	NS	NS

[a]Values are mean ± standard deviation (SD), except where noted.
[b]Frequency of sunflower kernel consumption was assessed by the response of each volunteer to a food frequency questionnaire.
[c]Because the data for these parameters did not follow a normal distribution, a natural logarithm transformation was performed before the ANOVA was run. These values represent the back transformed means plus 1 SD range.

Table 7. Whole blood (WB) cadmium, erythrocyte (RBC) cadmium, hemoglobin, serum ferritin, iron, zinc, copper, urea, and creatinine in humans consuming variable amounts of sunflower kernels[a]

	Frequency of sunflower kernel consumption[b]				*p*-Value determined by ANOVA		
	Women		Men				
	≤1 oz/week	>1 oz/week	≤1 oz/week	>1 oz/week	Sex	Group	Sex × group
Number	29	9	20	8			
Hematocrit (%)	39.0 ± 2.7	40.8 ± 1.6	44.2 ± 2.2	44.5 ± 3.2	<0.001	NS	NS
WB Cd (nmol/l)[c]	5.63 ± 2.53	6.15 ± 2.15	7.04 ± 2.29	7.79 ± 3.11	<0.04	NS	NS
RBC Cd (nmol/l)	14.3 ± 6.5	15.2 ± 5.4	15.9 ± 5.1	17.7 ± 7.5	NS	NS	NS
Hemoglobin (g/dl)	13.1 ± 0.9	13.4 ± 0.5	15.0 ± 0.8	15.0 ± 0.9	<0.001	NS	NS
Serum							
Ferritin (µg/l)[d]	31 (11–86)	39 (19–77)	79 (28–216)	90 (49–167)	<0.002	NS	NS
Fe (µmol/l)	16.2 ± 5.6	16.0 ± 5.3	19.5 ± 3.9	19.4 ± 4.7	<0.02	NS	NS
Zn (µmol/l)	12.3 ± 2.9	12.0 ± 1.2	14.1 ± 3.4	12.8 ± 1.9	NS	NS	NS
Cu (µmol/l)	17.0 ± 5.2	17.4 ± 2.8	14.8 ± 3.3	14.6 ± 2.9	<0.04	NS	NS
Urea (µmol/l)	4.7 ± 1.1	4.5 ± 0.7	5.2 ± 1.1	5.3 ± 0.9	<0.03	NS	NS
Creatinine (µmol/l)	77.9 ± 12.5	77.1 ± 9.4	94.1 ± 9.5	97.8 ± 12.6	<0.001	NS	NS

Abbreviations: ANOVA, analysis of variance; NS, not significant.
[a]Values are mean ± standard deviation (SD), except where noted.
[b]Frequency of sunflower kernel consumption was assessed by the response of each volunteer to a food frequency questionnaire.
[c]Whole blood cadmium was calculated based on the actual analysis of washed red cells and the value of individual hematocrit.
[d]Because the data for this parameter did not follow a normal distribution, a natural logarithm transformation was performed before the ANOVA was run. These values represent the back transformed means plus 1 SD range.

the higher amount of sunflower kernels ($p<0.02$). On average, the amount of NAG activity in this group was about 22% higher in women and 17% higher in men. However, when expressed as a function of urine creatinine, there were no significant differences. Expressed in this fashion, women excreted significantly more NAG than men ($p<0.002$).

Urinary β2MG is a small molecular mass protein that also is used to monitor kidney dysfunction. Although the variation in amounts excreted between subjects was relatively large, an analysis of the ln transformed data expressed on a urine volume basis showed that significantly more of the protein was excreted by volunteers consuming high amounts of sunflower kernels than by those consuming a lesser amount ($p>0.03$; Table 8). However, when β2MG was expressed as a function of creatinine concentration, there was no significant difference caused by sunflower kernel consumption ($p = 0.092$). On this basis, women tended to excrete more β2MG than men ($p = 0.055$).

Discussion

The primary objective of this study was to determine if we could detect a change in cadmium status of individuals who reported regular consumption of sunflower kernels that contained a natural concentration of cadmium, which is higher than in most other types of food. To make this determination, we used a battery of tests that have been used by other investigators to monitor dietary cadmium intake, cadmium concentrations in the body, and possible physiological effects of an excessive cadmium burden. These included food intake diaries to monitor cadmium intake, the concentrations of cadmium in RBCs and cadmium excretion in urine and feces to monitor body burden, and urinary proteins such as NAG and β2MG to monitor physiological effects.

Dietary cadmium intake was determined by database calculations of the cadmium content of foods reported by individuals in a 7-day food diary. Because the database underestimated cadmium content of the diets, the values were corrected by determining the differences between calculated and actual analyzed cadmium values in identical daily diets of one individual over a 7-day period. Although this method may seem somewhat inexact, the values obtained were comparable to those found by other investigators. Our control subjects who consumed few sunflower kernels had dietary cadmium intakes of approximately 36 ± 15 μg/day (mean ± SD). In a metabolic study by Spencer et al. (*8*), the analyses of 16 diets made up of natural foods showed a daily intake of 33 ± 1 μg cadmium. However, Berglund et al. (*9*) reported an average cadmium intake of only 14 ± 6 μg/day for 53 Swedish women consuming natural foods. The value of 20 μg cadmium/day, usually quoted for the typical intake of Americans, was derived primarily from the FDA Total Diet Study of Cadmium and reported by Gartrell et al. (*1*).

In our study, women who reported consuming >1 oz sunflower kernels/week had cadmium intakes not significantly different than the controls. However, men who reported consuming >1 oz sunflower kernels/week had cadmium intakes that were nearly twice as high as the controls. The difference between the intakes of men and women could have been due in part to the fact that six out of the eight men in the high intake group reported eating more than 1 oz sunflower kernels/day, while none of the nine women in the high intake group reported consuming that much.

Although the dietary intake of cadmium was significantly higher in some of those individuals reporting high consumption of sunflower kernels, there were few changes in the cadmium status monitoring indices. RBC or whole blood concentrations of cadmium and urinary excretion of cadmium were not affected by high consumption of sunflower kernels. Whole blood cadmium across kernel intakes ranged from 0.14 to 1.29 μg/l in females and 0.33 to 1.27 μg/l in males. These values were similar to those reported for whole blood cadmium by others. Iyengar and Woittiez (*10*) reported a range of 0.3 to 7.0 μg/l for whole blood cadmium in 53 nonsmokers. These data were based on a worldwide survey of reliable laboratory values, but sex was not specified. Berglund et al. (*9*) reported a range of <0.09–0.96 μg of cadmium/l whole blood of nonsmoking Swedish women. These investigators also showed a highly significant positive correlation between the concentrations of serum ferritin and blood cadmium, but we could not demonstrate a correlation in our sampling of subjects (correlations not shown). Vahter et al. (*11*) reported that women who increased their cadmium intake twofold (from 10 to 22 μg/day) by supplementing their diet with shellfish did not have a significant increase in blood cadmium.

Based on the accuracy of cadmium determination in the quality control standards run concurrently with our analysis, we assume that our values for blood cadmium are correct. If so, we conclude that nonsmoking individuals living in the northern Great Plains region of the United States and consuming large amounts of sunflower kernels with higher than average food cadmium have blood cadmium concentrations that are no greater than those individuals who consume little or no sunflower kernels.

Other markers for excessive cadmium exposure include the excretion of cadmium in urine and feces. We found that individuals in our study excreted about 1.5 μg of cadmium/l urine. Although we did not collect 24-hr urine, we estimate that the cadmium output was approximately 2.5 μg/day, based on an average daily urine output for men and women of this age group. Iyengar and Woittiez (*10*) reported a range of urinary cadmium of 0.5–4.7 μg/l, which is similar to what we found. However, Berglund et al. (*9*) reported urine cadmium values in women that were only one-tenth of the values we found. It is not apparent why their values are so much lower than ours, except that cadmium intakes of their subjects were less than half of that reported by our subjects. Vahter et al. (*11*), on the other hand, reported that women who increased their cadmium intake

Table 8. Cadmium, creatinine, *N*-acetyl-β-D-glucosaminidase (NAG) activity, and β_2-microglobulin (β2MG) in urine of humans consuming variable amounts of sunflower kernels[a]

	Frequency of sunflower kernel consumption[b]				*p*-Value determined by ANOVA		
	Women		Men				Sex ×
	≤1 oz/week	>1 oz/week	≤1 oz/week	>1 oz/week	Sex	Group	group
Number	29	9	20	8			
Cd (nmol/l)	13.2 ± 6.6	12.2 ± 7.9	16.1 ± 8.1	15.0 ± 7.6	NS	NS	NS
Cd (nmol/g) creatinine	15.0 ± 9.1	11.9 ± 5.1	11.6 ± 7.7	7.9 ± 3.5	NS	NS	NS
NAG (U/l)	1.7 ± 0.7	2.2 ± 0.8	1.9 ± 0.7	2.3 ± 0.6	NS	<0.02	NS
NAG (U/g creatinine)	1.8 ± 0.7	2.2 ± 0.6	1.4 ± 0.7	1.4 ± 0.6	<0.002	NS	NS
β2MG (μg/l)[c]	20 (9–46)	41 (21–80)	19 (5–73)	36 (10–129)	NS	<0.03	NS
β2MG (μg/g creatinine)[c]	22 (9–53)	43 (17–104)	13 (3–57)	20(6–72)	0.055	0.092	NS
Creatinine (g/l)	1.01 ± 0.47	1.07 ± 0.49	1.56 ± 0.57	1.91 ± 0.67	<0.001	NS	NS

ANOVA, anaysis of variance.
[a]Values are mean ± standard deviation (SD), except where noted.
[b]Frequency of sunflower kernel consumption was assessed by the response of each volunteer to a food frequency questionnaire.
[c]Because the data for these parameters did not follow a normal distribution, a natural logarithm transformation was performed before the ANOVA was run. These values represent the back transformed means plus 1 SD range.

twofold (from 10 to 22 µg/day) by supplementing their diet with shellfish, did not have an increase in urine cadmium.

Because the absorption of cadmium has been shown to range from 2 to 10%, the amount of cadmium excreted in the feces each day should closely reflect the amount of cadmium consumed. This was well demonstrated by Berglund et al. (*9*); daily fecal cadmium excretions were almost identical to the amounts ingested. Viewed in conjunction with their low urine output data, there was very little cadmium absorbed by subjects in these studies. Data from our study did not show the close association between cadmium intake and fecal output. Only about 60% of the cadmium ingested was excreted in the feces over the collection period used. The imprecise method of determining cadmium intake may have accounted for part of this discrepancy in our study.

Cadmium absorption depends on the composition of the diet and the nutritional status of an individual. Flanagan et al. (*12*) showed that cadmium absorption as a percent of intake was inversely proportional to the iron status of individuals. Those with high serum ferritin absorbed about one-fourth as much cadmium as those with low serum ferritin. Berglund et al. (*9*) also showed that blood cadmium concentrations were significantly higher in women who had serum ferritin concentrations of <20 µg/l compared to those with >30 µg/l. However, they could find no significant correlation between food cadmium intake and whole blood cadmium; they concluded that blood cadmium may not be a useful indicator of low-level exposure to cadmium. Although women in the present study had lower serum ferritin concentrations than men, there was no indication that whole blood and RBC cadmium concentrations were elevated.

Other dietary factors affect cadmium availability. These include zinc (*13*), copper (*14*), fiber (*9*), and phytate (*15*). An increased intake of sunflower kernels not only will increase cadmium intake but also the intake of copper and phytate. In turn, this could reduce the availability of cadmium from this food source. Reeves et al. (*16*) showed that cadmium availability to rats fed purified diets containing 20% ground sunflower kernels was less than that from similar diets not containing kernels. They contributed part of this reduction in availability to the elevated phytate content of the diet. An increase in copper intake as a result of consuming sunflower kernels, as shown in the present study, also could contribute to less cadmium availability from sunflower kernels.

Elevated NAG activity and the amount of β2MG in urine are generally accepted as good indicators of excessive cadmium exposure. NAG activity has been shown to increase in a variety of renal diseases (*17,18*) and has been observed to be higher in individuals exposed to cadmium-polluted areas (*19*) and to cadmium in the work-place (*20,21*). In the present study, when NAG excretion was expressed as units per liter, there was significantly more in the urine of volunteers who reported consuming large amounts of sunflower kernels than in those who reported low consumption. However, when the values were expressed as units per gram creatinine, there was no difference. Kawada et al. (*22*) showed a significant correlation between urinary cadmium and NAG excretion, and the association appeared in cadmium-exposed populations whose urinary cadmium concentrations were as low as 2 µg/g creatinine. Urinary cadmium excretion of the subjects in our study ranged from 0.3 to 2.0 µg/g creatinine (1.4 ± 0.9, mean ± SD), but we could not show a significant correlation between urinary cadmium and excretion of this protein (data not presented).

Results similar to those for urinary NAG activity were found for the urinary excretion of β2MG as well. Data from the National Health and Nutritional Examination Survey II (NHANES II) survey on cadmium and reported by Kowal and Zirkes (*23*) showed that adults in the age range represented in our study, and not exposed to environmental cadmium, had urine β2MG concentrations (microgram per gram creatinine) that were 60% higher than those in our control volunteers who reported consuming very little if any sunflower kernels. The use of β2MG and NAG as useful monitors for cadmium exposure has been questioned because 1) β2MG is notoriously unstable, both β2MG and NAG are affected by age, and the variability between subjects is high; and 2) a normal range of excretion has not been clearly established. Therefore, although we observed a small increase in urine protein markers in subjects who consumed high amounts of sunflower kernels, they were not outside the normal ranges found by other investigators (*23,24*). In addition, because we could not distinguish between subjects who ate sunflower kernels with low amounts of cadmium from those who ate kernels with very high amounts, there was no way to determine if the consumption of the kernels themselves did not cause the apparent rise in these protein markers.

Whether the present findings indicate that elevated, long-term consumption of low levels of cadmium from sunflower kernels is potentially hazardous should be weighed against the limitations of this study. First, and perhaps the most important, is the small number of subjects in each group. A sample number analysis based on the variance of the urinary protein variables showed that in order to obtain a 25% difference between groups with a power of 0.8, we would need approximately 30 subjects/group. Unfortunately, it was very difficult to obtain this many subjects who report high consumption of sunflower kernels. The sunflower kernel is a speciality food and is not consumed by a large majority of the population. Women in general do not eat high amounts of sunflower kernels, perhaps because of their high caloric content. We identified specific groups of men who were likely to consume sunflower kernels. These groups included baseball and softball players, delivery and long distance drivers, and line workers in sunflower kernel processing plants. However, we could not persuade them to volunteer for the study, mainly because they were opposed to collecting fecal and urine samples on the job.

Another inadequacy of this study was the reliance on calculated values to estimate the dietary intake of cadmium. Most nutrient databases do not have reliable values for cadmium in most foods. We could have collected duplicate diets and analyzed them for cadmium, but again, because of personal constraints of many of the potential volunteers, especially the high kernel consumers, compliance would not have been adequate. In addition, at the time this study was done, this procedure would have been prohibitively expensive and time consuming for our laboratory. The alternative method was to correct the intake values based on a limited number of actual diet analyses. As a result, individual daily cadmium intakes, although close to what others have found, were estimates at best. Nonetheless, with these limitations in mind, it is evident that with the present concentration of natural cadmium in sunflower kernels, a very high habitual consumption of the kernels will lead to an elevated weekly intake of food cadmium. Whether this in turn will eventual lead to long-term health effects is still to be determined, and these determinations should be pursued with controlled human feeding studies.

References

1. Gartrell MJ, Craun JC, Podrebarac DS, Gunderson EL. Pesticides, selected elements, and other chemicals in adult total diet samples, October 1980–March 1982. J Assoc Off Anal Chem 69:146–159 (1986).
2. Pennington JAT, Wilson DB, Newell RF, Harland BF, Johnson RD, Vanderveen JE. Selected minerals in food surveys, 1974 to 1981/82. J Am Diet Assoc 84:771–780 (1984).
3. Li Y-M, Chaney RL, Schneiter AA, Miller JF. Genotypic variation in kernel cadmium concentration in sunflower germplasm under varying soil conditions. Crop Sci 35:137–141 (1995).

4. Chaney RL, Ryan JA, Li Y-M, Welch RM, Reeves PG, Brown SL, Green CE. Phyto-availability and bio-availability in risk assessment for cadmium in agricultural environments. In: Sources of Cadmium in the Environment. Paris:Organization for Economic Co-operation and Development, 1996;49–78.
5. WHO. Evaluation of Certain Food Additives and Contaminants. 33rd Report of the Joint FAO/WHO Expert Committee on Food Additives 776. Geneva:World Health Organization, 1989;28–31.
6. Dieter M. Rapid colorimetric assay of β-galactosidase and *N*-acetyl-β-glucosaminidase in human urine. Clin Chim Acta 73:453–461 (1976).
7. Tukey JW. Comparing individual means in the analysis of variance. Biometrics 5:99–114 (1949).
8. Spencer H, Asmussen CR, Holtzman RB, Kramer L. Metabolic balances of cadmium, copper, manganese, and zinc in man. Am J Clin Nutr 32:1867–1875 (1979).
9. Berglund M, Åkesson A, Nermell B, Vahter M. Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. Environ Health Perspect 102:1058–1066 (1994).
10. Iyengar V, Woittiez J. Trace elements in human clinical specimens: evaluation of literature data to identify reference values. Clin Chem 34:474–481 (1988).
11. Vahter M, Berglund M, Nermell B, Åkesson A. Bioavailability of cadmium from shellfish and mixed diet in women. Toxicol Appl Pharmacol 136:332–341 (1996).
12. Flanagan PR, McLellan JS, Haist J, Cherian MG, Chamberlain MJ, Valberg LS. Increased dietary cadmium absorption in mice and human subjects with iron deficiency. Gastroenterol 74:841–846 (1978).
13. Evans GW, Magors PF, Cornatzer WE. Mechanism for cadmium and zinc antagonism of copper metabolism. Biochem Biophys Res Commun 40:1142–1148 (1970).
14. Bremner I, Campbell JK. The influence of dietary copper intake on the toxicity of cadmium. Ann NY Acad Sci 355:319–332 (1980).
15. Wing AM. The effects of whole wheat, wheat bran and zinc in the diet on the absorption and accumulation of cadmium in rats. Br J Nutr 69:199–209 (1993).
16. Reeves PG, Johnson PE, Rossow KL. Absorption and organ content of cadmium from the kernels of confectionery sunflowers (*Helianthus annuus*) fed to male rats. J Agric Food Chem 42:2836–2843 (1994).
17. Dance N, Price RG. The excretion of *N*-acetyl-β-glucosaminidase and β-glactosidase by patients with renal disease. Clin Chim Acta 27:87–92 (1970).
18. Wellwood JM, Ellis BG, Price RG, Hammond K, Thompson AE, Jones NF. Urinary *N*-acetyl-β-D-glucosaminidase activity in patients with renal disease. Br Med J 3:408–411 (1975).
19. Nogawa K, Yamada Y, Kido T, Honda R, Ishizaki M, Tsuritani I, Kobayashi E. Significance of elevated urinary *N*-acetyl-β-D-glucosaminidase activity in chronic cadmium poisoning. Sci Total Environ 53:173–178 (1986).
20. Bernard A, Thielemans N, Roels H, Lauwerys R. Association between NAG-B and cadmium in urine with no evidence of a threshold. Occup Environ Med 52:177–180 (1995).
21. van Sittert NJ, Ribbens PH, Huisman B, Lugtenburg D. A nine year follow up study of renal effects in workers exposed to cadmium in a zinc ore refinery. Br J Ind Med 50:603–612 (1993).
22. Kawada T, Shinmyo RR, Suzuki S. Urinary cadmium and *N*-acetyl-β-D-glucosaminidase excretion of inhabitants living in a cadmium-polluted area. Int Arch Occup Environ Health 63:541–546 (1992).
23. Kowal NE, Zirkes M. Urinary cadmium and β_2-microglobulin: normal values and concentration adjustment. J Toxicol Environ Health 11:607–624 (1983).
24. Strehlow CC, Barltrop D. Health studies. The Shipham Report: an investigation into cadmium contamination and its implications for human health. Sci Total Environ 75:101–133 (1988).